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ORIGINAL PAPER

Simultaneous removal of nutrients in a novel anaerobic–anoxic/aerobic sequencing reactor: removal of nutrients in a novel reactor

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Abstract Discharging wastewater contaminated with nitrogen and phosphorus compounds (nutrients) can be harmful to both human health and the environment. The present study investigated the efficiency of removing total nitrogen, total phosphate, and chemical oxygen demand using a novel reactor design. The reactor was an anaerobic–anoxic/aerobic sequencing batch reactor with continuous influent and discontinuous outlet. The operation consisted of four phases. Phases 3 and 4 had mixing cycles, whereas phases 1 and 2 only had aeration, settling, and discharge cycles. Phase 4 was the most effective. The average removal efficiencies for chemical oxygen demand, NH_4^+, total nitrogen, and total phosphate in Phase 4 were 92, 91, 86, and 85 %, respectively. This phase comprised 225 min of intermittent aeration time, 165 min of intermittent mixing time, and 90 min of settling and discharge. In addition to high efficiency in nutrient removal, the reactor had other advantages, including continuous flow and performance in all phases.

Keywords Nitrogen · Phosphate · Sequencing batch reactor · Wastewater

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Introduction

The discharge of wastewater contaminated with nitrogen and phosphorus compounds (nutrients) as a result of human activities can have harmful effects on human health and the environment. Excessive nutrients in water bodies can cause a variety of problems, including reduced oxygen concentration in water, which can lead to fish death, eutrophication, and over-fertilization. Eutrophication is associated with a range of water quality issues such as increased water treatment costs, reduced recreation quality, and the production of algal toxins with potentially lethal effects on humans (Falkowski et al. 2000; Morse et al. 1998; Renman et al. 2008).

A variety of physicochemical, chemical, and biological methods have been used to remove nutrients from wastewater. Phosphorus removal is possible by physicochemical methods (deposition using materials such as alum, lime, and iron salts) and biological methods (Phostrip, Bardenpho, A/O) (Morse et al. 1998). Removal of nitrogen compounds from wastewater has been performed through physical methods (reverse osmosis, air stripping), chemical methods (ion exchange, breakpoint chlorination), and biological methods (nitrification–denitrification) (Renman et al. 2008). Among these approaches, biological nutrient removal has been considered the most economical and efficient method of wastewater treatment (Li and Irvin 2007; Sibag and Kim 2012; Yuan et al. 2012).

Biological nitrogen removal involves two processes, namely nitrification and denitrification, whose final product is nitrogen gas. First, nitrification is performed by autotrophic microorganisms in aerobic conditions. Then, denitrification is performed in anoxic conditions by heterotrophic microorganisms (Fu et al. 2009a, b; Wei et al. 2012; Wu et al. 2014). In the anaerobic phase, phosphate-

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accumulating organisms (PAOs) sequester organic substances, especially volatile fatty acids (VFAs), in their cells as polyhydroxyalkanoates (PHAs). In the aerobic phase, PHA acts as an energy source for the uptake of phosphate from solution. Finally, the phosphate-rich sludge is discharged from the treatment system. Phosphate adsorption above cellular capacity by PAOs is possible in enhanced biological phosphorus removal (EBPR). EBPR places the PAOs in alternating anaerobic and aerobic conditions (Zhou et al. 2012; Drewnowski and Makinia 2014). Simultaneous removal of total phosphate (TP) and total nitrogen (TN) consumes organic matter. However, the chemical oxygen demand (COD) of the denitrifier microorganisms leads to problems in the EBPR process because it reduces the efficiency of the PAOs in the uptake and storage of the organic substances needed for absorption of the phosphate. The formation of nitrate by the microorganisms in the aerobic phases and its presence in the anaerobic phases blocks phosphate release by the PAOs. A sequencing batch reactor (SBR) allows control of the environment in terms of electron receptors (oxygen or nitrate) and is therefore a good option for nutrient removal. Its benefits include control of oxygen levels and redox conditions, use of aerobic and anaerobic cycles, and low operating costs (Fu et al. 2009b). The SBR process has been widely used for simultaneous removal of COD and nutrients (Azhdarpoor et al. 2014; Aslan and Kapdan 2006; Cui et al. 2014). In general, simultaneous removal of nutrients in a single reactor is advantageous because it avoids operational complexities (Park et al. 2009; Wei et al. 2012). As noted, to remove both nitrogen and phosphorus compounds, alternating anaerobic and aerobic conditions are required, which are present in the SBR process. However, disruptions caused by denitrifiers have required many refinements to SBR systems (Fu et al. 2009b). The reactor system used in this study was designed to tackle this. The study aimed to investigate the simultaneous removal of nutrients (TN and TP) and COD from wastewater using a novel anaerobic–anoxic/aerobic SBR. The advantages of this modified anaerobic/aerobic SBR reactor include high efficiency and inclusion of anaerobic and aerobic tanks in a single reactor, thus reducing the space needed. Also, unlike conventional SBR reactors, the influent is continuous and upward moving. The research was carried out at the main environmental sciences laboratory of Shiraz University of Medical Sciences.

Materials and methods

Operational phases

This study consisted of four phases. In phases 1 and 2, aeration and settling cycles were used, while phases 3 and 4 used alternating aeration, mixing, and settling cycles. Phases 1, 2, 3, and 4 were run for 180, 240, 360, and 480 min, respectively. Details of the operational phases are presented in Fig. 1.

Synthetic wastewater

Synthetic wastewater was used to avoid fluctuations in the amounts of COD, ammonia, and phosphate in the influent and to maintain control over the reactor system (Rahimi et al. 2011). The minimum and maximum inlet concentrations were 250 and 1500 mg/l COD, 4 and 61 mg/l TP, and 40 and 100 mg/l ammonia. The pH level of the inlet wastewater was approximately 7.4. The water required to produce the synthetic wastewater was taken from the urban water supply network. The materials and values used to produce the synthetic wastewater are shown in Table 1.
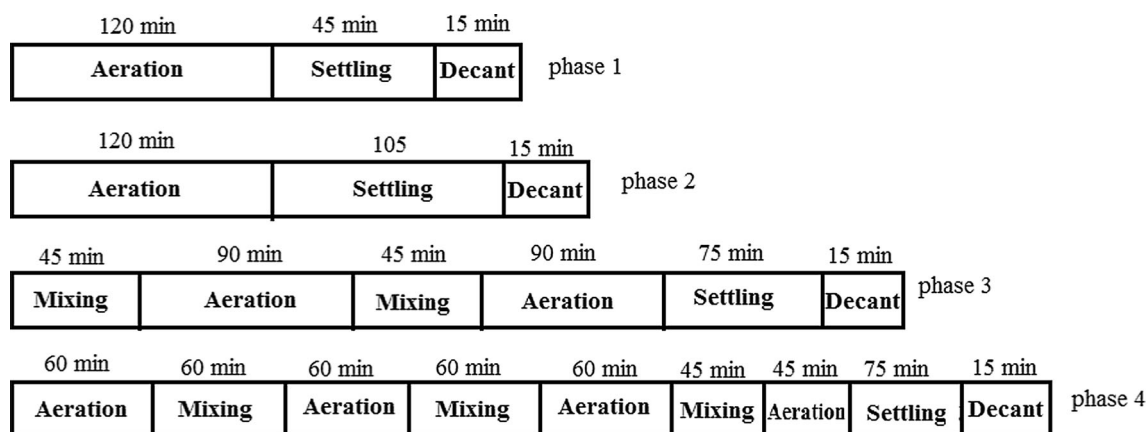

Fig. 1 Four operational phases in an anaerobic–anoxic/aerobic sequencing batch reactor

Table 1 Characteristics of the influent to the reactor

Material	Concentration (g/l)
Glucose	0.33–0.97
Sucrose	0.12–0.36
Sodium acetate	0.12–0.36
KH_2PO_4	0.011–0.84
NH_4Cl	0.14–0.39
Trace elements (mg/l)	
$ZnSO_4$	0.45
$FeCl_3$	0.1
Na_2MoO_4	0.8
$CoCl_2$	0.4
$CuSO_4$	0.4
$NiCl_2$	0.2
$MnCl_2$	0.28

Reactor description

A modified form of SBR was used in this study. The synthetic wastewater entered the reactor continuously in an upward flow, and the effluent was discharged discontinuously. The reactor consisted of two parts. The first part was permanently anaerobic. The second part, which was anoxic/aerobic, was separated by a Plexiglas sheet from the anaerobic part. These conditions were maintained by aeration and the use of an electric timer. To achieve mixing in the absence of aeration in the anoxic/aerobic phases 3 and 4, a mixer was used with the engine speed kept constant at 50 rpm. The reactor had an internal diameter of 15 cm and a height of 40 cm. The total reactor volume was 9 l and the working volume ranged from 5 l in Phase 1 to 7 l in the Phase 4. A schematic presentation of the reactor and its accessories is shown in Fig. 2.

Reactor operation

First, return sludge from the aeration tank of the wastewater treatment plant of Shiraz, Iran, was used for microbial seeding of the system. No samples were taken during the first 14 days of the study, to allow the microorganisms to adapt to conditions different from those of the treatment plant. The duration of reactor operation was 150 days. The concentration of dissolved oxygen (DO) was about 3 mg/l in the aerobic cycle, around 0–0.2 mg/l in the mixing cycle, and 0 mg/l in the settling cycle. A DO meter (HQd Field Case, Hach, USA) was used to measure DO, and a pH meter (Metrohm model 826, Switzerland) was used to measure pH. For controlling aeration, mixing, and discharge, three electric timers (Theben-Germany) were used. A solenoid valve (2&2, China) was used for discontinuous discharge of the supernatant. Finally, an aquarium air pump was used for aeration of the system (Leviton, USA).

Testing methods

COD was measured following standard methods (APHA 1999). A spectrophotometer (model DR-5000, Hach) was used to measure TP, ammonium (NH_4^+), nitrate (NO_3^-),
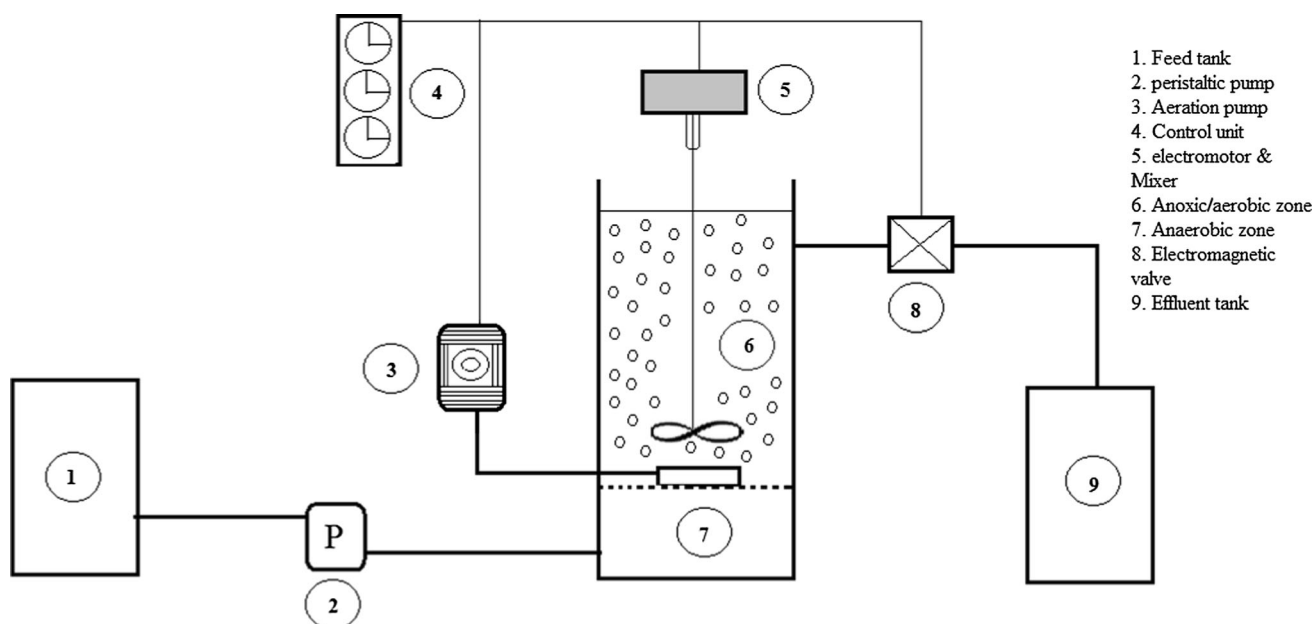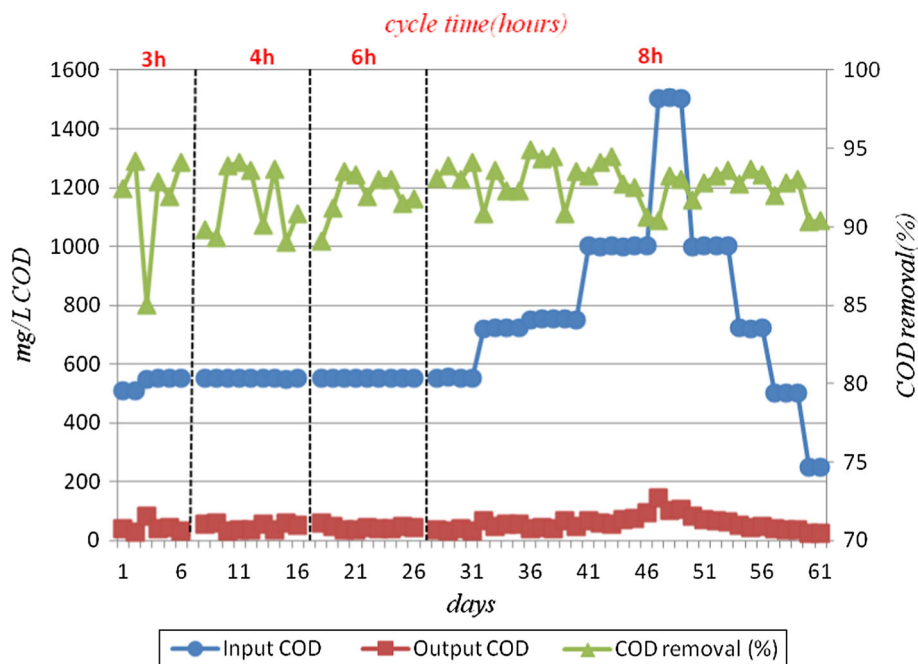

1. Feed tank
2. peristaltic pump
3. Aeration pump
4. Control unit
5. electromotor & Mixer
6. Anoxic/aerobic zone
7. Anaerobic zone
8. Electromagnetic valve
9. Effluent tank

Fig. 2 Schematic presentation of a novel anaerobic–anoxic/aerobic sequencing batch reactor and its accessories

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Fig. 3 Input COD removal in four phases of an anaerobic–anoxic/aerobic sequencing batch reactor

Results and discussion

COD removal

COD removal efficiency was high in all phases, and no significant differences were observed between different phases (Fig. 3). Phases 1, 2, and 3 had inlet COD values of 550 mg/l, and none could be considered more efficient than the others. Phase 4, with COD concentrations up to 1500 mg/l, was superior to the other phases. Figure 3 shows that there was very little fluctuation in the removal efficiency curve as COD increased; a constant smooth reduction was observed to the end of the phase. This suggests that the reactor had a tolerance for high levels of COD and COD shock loading. The average COD removal efficiency was 91.7, 91.5, 92, and 92.7 % in phases 1, 2, 3, and 4, respectively. Most nutrient removal systems are effective in reducing COD, because organic materials are necessary in nutrient removal processes and much of the COD takes place in anaerobic conditions (Fu et al. 2009a; Chae and Shin 2007). In systems for simultaneous removal of nutrients and COD, a large amount of COD is consumed by the denitrifiers and PAOs. Fu et al. 2009a investigated simultaneous nitrogen and phosphorus removal using a novel sequencing batch moving bed membrane bioreactor (SBMBMBR). At inlet COD concentration of 500 mg/l, they found an average COD removal efficiency of 93.5 % (Fu et al. 2009a). In our

and nitrite (NO_2^-). In the measurement of TP using the ascorbic acid method (No. 8048), orthophosphate in the sample reacts with molybdate in an acidic medium and a complex is formed. Ascorbic acid reduces this complex, and a blue color is created, the intensity of which represents the amount of orthophosphate in the sample. The measurement wavelength is 880 nm for spectrophotometry. In the measurement of ammonia by the Nessler method (No. 8038), polyvinyl alcohol (as dispersing agent) produces a yellow color during the reaction of the Nessler reagent with ammonia and certain other amines. This can be detected at a wavelength of 425 nm. In the measurement of nitrate by the cadmium reduction method (No. 8039), metal cadmium converts the nitrate in the sample into nitrite. Nitrite ions in the sulfanilic acidic medium react to form diazonium salt. The salt then reacts with gentisic acid, and an amber solution is formed. This can be detected at a wavelength of 520 nm. In nitrite measurement *by the USEPA* Diazotization Method (No. 8507), the nitrite present in the sample reacts with sulfanilic acid and a diazonium salt is formed. This salt reacts with chromotropic acid, and a pink complex is formed which can be detected at a wavelength of 507 nm (Wang et al. 2010). All the experimental reagents used in the present study were of analytical grade. The average values of the data obtained under steady state conditions were used for further calculations (at 5–7 days for each concentration).
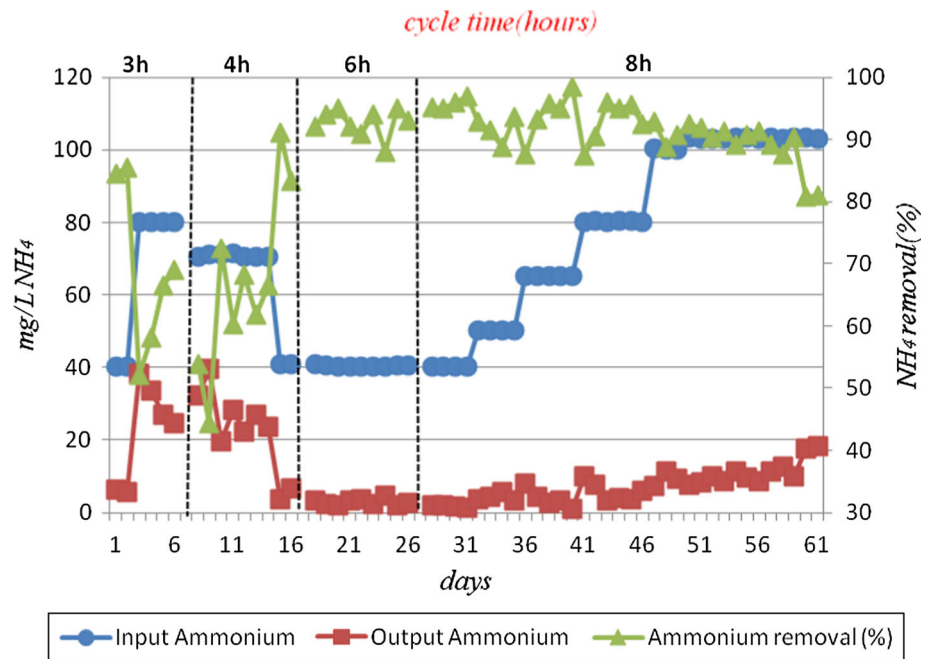
Fig. 4 Ammonia removal in four phases of an anaerobic–anoxic/aerobic sequencing batch reactor


Removal of nitrogen compounds

To remove ammonia, two stages of aerobic nitrification and anoxic denitrification are required. Oxygen concentrations reported in different studies range from 2 to 5 mg/l in the first stage and are below 0.3 mg/l in the second (Hu et al. 2010) (Fig. 4). In the current study, the value was 3.5 in aeration conditions, falling to near zero in anoxic conditions. Moreover, according to Luostarinen et al. (2006), anaerobic pretreatment produces compounds capable of further decomposition, which are useful for biological removal of nitrogen compounds. Our reactor also demonstrated this advantage. With an input of 40 mg/l ammonia at the beginning of Phase 1, a removal efficiency of 85 % was observed. As the input concentration increased to 80 mg/l NH_4^+, this efficiency fell to 61 %, showing that Phase 1 had peak efficiency at inlet concentrations of 40 mg/l NH_4^+. Similar results can be seen in Fig. 5 for TN removal. Nitrate output was initially at about 14 mg/l and then increased to 26 mg/l. Similarly, nitrite was initially 0.87 mg/l, increasing to 8.3 mg/l. In Phase 2, fluctuations were observed in NH_4^+ and TN removal efficiency at inlet ammonia concentrations of 70 mg/l. The average removal efficiency reached 61 % for NH_4^+ and 52 % for TN. The average concentration of output nitrate and nitrite was 16 and 5 mg/l, respectively. However, the concentration of input NH_4^+ was 40 mg/l, a higher removal efficiency than in Phase 1 (approximately 90 %). At this concentration,

TN removal efficiency was above 52 % and output nitrate and nitrite concentrations were 8.5 and 12 mg/l, respectively. In their study on the efficiency of biological nutrient removal in an SBR, Kargi and Uygur found that shorter anoxic/aerobic periods led to better nutrient removal efficiency (Kargi and Uygur 2003). Freitas studied the effect of short SBR cycles on nutrient removal and found many advantages of short cycles, including increased efficiency of nutrient removal, improved SBR process performance, reduced energy consumption, and increased richness of anaerobic and aerobic microbes (Freitas et al. 2009). In a study of nitrogen and phosphorus removal from abattoir wastewater in an SBR with aerobic granular sludge, shorter settlement cycles in the SBR process were found to improve waste removal (Cassidy and Belia 2005). In the present study, Phase 3 coincided with the entry into operation of the reactor and the use of short anoxic/aerobic cycles with a shorter settling time. During this phase, NH_4^+ removal efficiency remained at 90 % ± 2. This consistency was also observed for TN removal efficiency. In Phase 3, the average TN removal efficiency was 71 % and the amounts of nitrate and nitrite discharged from the reactor were 21 and 6.5 mg/l, respectively. Phases 2 and 3 were similar in terms of NH_4^+ input (40 mg/l). However, Phase 3 had higher efficiency and its curve showed smaller fluctuations. Thus, Phase 3 was superior to Phase 2 in this regard. TN removal efficiency was 52 % in Phase 2 and 71 % in Phase 3. The beginning of Phase 4 was similar to Phase 3 in terms of NH_4^+ input (40 mg/l) with shorter cycles. Yet the average removal efficiency of NH_4^+ in Phase 4 was slightly higher (95 %). TN removal efficiency

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Fig. 5 Total nitrogen (TN) removal efficiency and nitrate and nitrite production in four phases of an anaerobic–anoxic/aerobic sequencing batch reactor
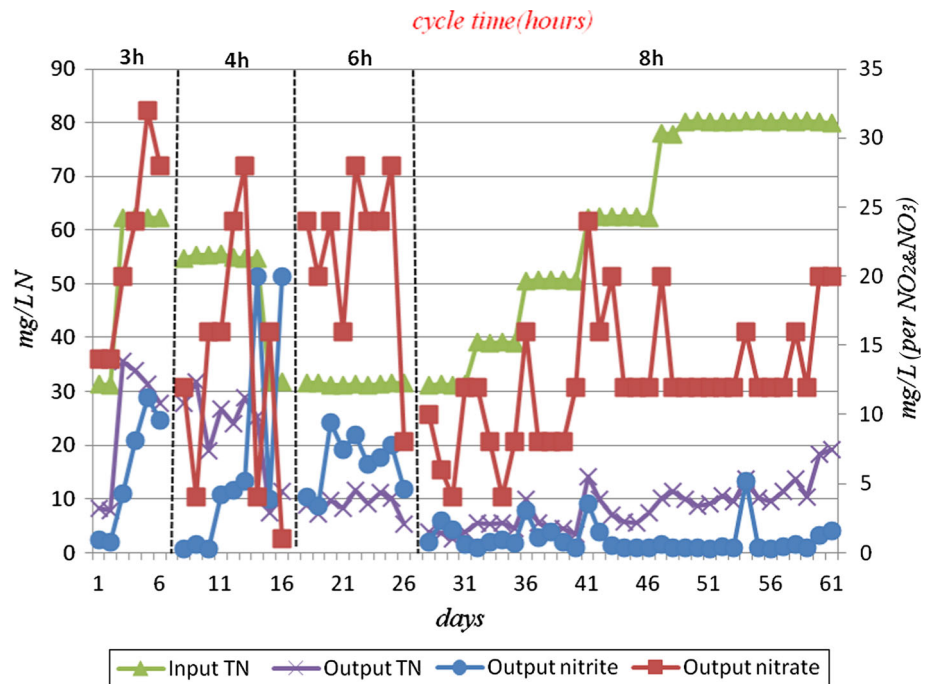
was 88.5 %, which is consistent with the results of a study by Li et al. (2012) on simultaneous removal of carbon and nitrogen in a circulating fluidized bed biofilm reactor (CFBBR). The study found that over 85 % of TN was removed during slow bioparticle circulation between Riser (Anoxic) and Downer (Aerobic) with 350 mg/l COD and 40 mg/l NH_4^+. Our reactor achieved similar efficiency levels without return sludge in terms of the amount of NH_4^+ input and COD (500 mg/l). Phase 4 was more efficient than Phase 3 in terms of TN removal; the amount of nitrate and nitrite discharged initially were 8 and 0.2–1.5 mg/l, respectively. In this phase, the amount of input NH_4^+ increased from 50 to 80 mg/l and COD increased from 720 to 1000 mg/l. The amount of input NH_4^+ and COD increased and NH_4^+ and TN removal efficiency increased to 93 and 87.7 %, respectively, in a stepwise manner. The amounts of output nitrate and nitrite were 12 and 1 mg/l, respectively. When holding NH_4^+ constant at 100 ± 2 mg/l and increasing the input COD to 1500 mg/l, NH_4^+ and TN removal efficiencies reached 91 and 86.5 %, respectively, while output nitrate and nitrite reached 14 and 0.44 mg/l. However, with consolidation of NH_4^+, the input COD value reduced from 1500 to 250 mg/l (C/N = 3.1–19). In other words, the reactor's performance was tested in the absence of carbon input for reducing the levels of NH_4^+. NH_4^+ and TN removal efficiencies remained at 90 and 87 %, respectively, up to an input COD of 500 mg/l, while output nitrate and nitrite levels were 12.8 and 0.8 mg/l, respectively. At an input COD of 250 mg/l, NH_4^+ and TN removal efficiency reached 81 and 77 %, respectively, and nitrate and nitrite

output reached 20 and 1.3 mg/l, respectively. This suggested that NH_4^+ removal efficiency in this phase was not significantly related to the input ammonia/carbon ratio. The highest removal efficiency (86.5 %) was recorded at a C/N ratio of 19. When the C/N ratio was reduced to 12.4, NH_4^+ removal efficiency fell to 80 %. The lowest ratio of C/N was 3.1, corresponding to an NH_4^+ removal efficiency of 80 %. The input COD removal efficiency did not change as input C/N ratios were reduced. Using real-time control with step-feed SBR technology, Guo reported that at a C/N ratio of 3.5, an input NH_4^+ concentration of 40–60 mg/l, and a COD input of 180–220 mg/l, TN removal efficiency reached 98 % (Guo et al. 2007). In our reactor, with continuous feeding and a C/N ratio of 19 (with an input COD of 1500 mg/l and NH_4^+ of 100 mg/l), TN removal efficiency was 86.5 %.

Removal of total phosphate

Because PAOs tend to use organic materials with low molecular weight, the anaerobic area at the beginning of the process plays a selective role in breeding PAOs (Metcalf and Eddy 2003). In phases 1 and 2, and at TP concentration of 8–16 mg/l, the removal efficiency was 58.1 and 84.5 %, respectively. In Phase 3, the value was 83.9 % at a concentration of 8 mg/l, and in Phase 4, it was 84.6 % at a concentration of 8–60 mg/l. At entry levels of 8 mg/l TP in Phase 1, the removal efficiency was approximately 70 % (Fig. 6). However, when the input TP was doubled, the removal efficiency reduced to approximately 50 %. This shows the inefficiency of Phase 1, which can be

Fig. 6 TP removal in four phases of an anaerobic–anoxic/aerobic sequencing batch reactor
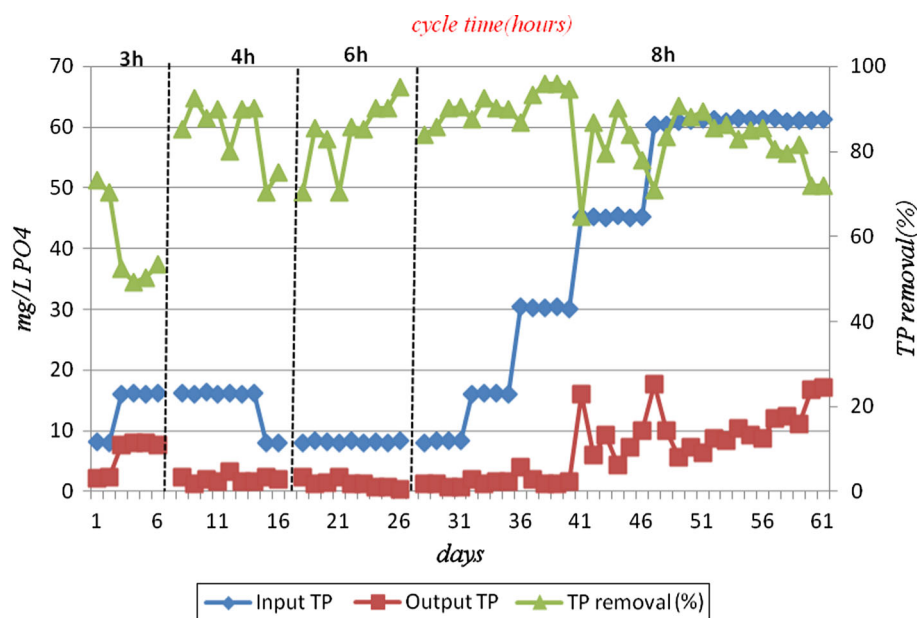
attributed to insufficient settling time (anaerobic). This limits the ability of the PAOs to obtain energy from organic materials to drive the uptake of phosphate in the following aerobic cycles (Yuan et al. 2012). The 100 % increase in settling time (anaerobic) in Phase 2 improved TP removal efficiency. In Phase 2, since conditions in the reactor were permanently anaerobic and the settling time was higher than that in Phase 1, the PAOs were able to gain energy by COD. This was reflected in the increase in TP removal efficiency to 84.5 %. At the end of Phase 2, the input TP of 8 mg/l was similar to that in Phase 3. However, the removal efficiency was higher in Phase 3. At the beginning of Phase 4, with inputs of 8 mg/l TP, the removal efficiency showed no significant increase from Phase 3. Phase 4 was tested with input TP concentrations of approximately 8, 16, 30, 45, and 60 mg/l. At input concentrations up to 30 mg/l, the removal efficiency was above 90 %. However, at an input concentration of 45 mg/l, the removal efficiency curve fluctuated more than at previous concentrations. At the end of Phase 4, when the input TP value was fixed at 60 mg/l, the level of COD decreased from 1500 to 250 mg/l. TP removal efficiency was also examined with decreasing amounts of input COD (Fig. 7). As the input COD was reduced, the phosphate removal efficiency remained acceptable until the input COD concentration reached the lowest level. This demonstrates the high removal efficiency of the reactor at very high concentrations of TP, even when input COD levels were very low; reducing the input COD/P ratio had very little impact on TP removal efficiency. This is an advantage of the new reactor design. The maximum and minimum values of COD/P ratio were 25 and 4.1, respectively, which were consistent with the input
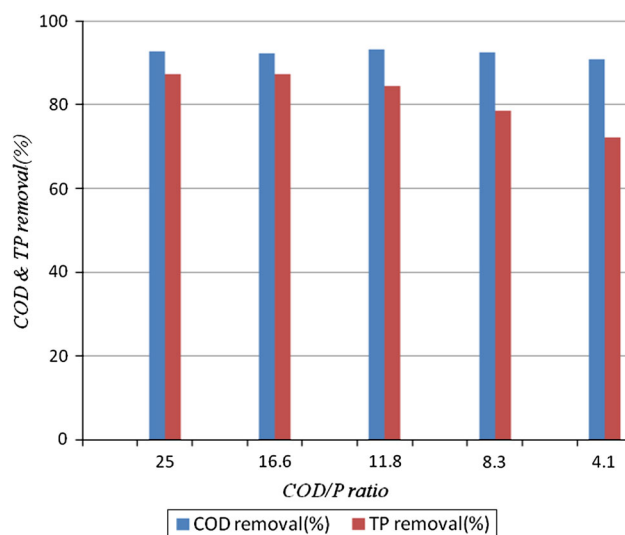

Fig. 7 COD/P ratio against TP and COD removal efficiency in Phase 4

COD of 250 mg/l. Across the entire range of COD/P ratios, COD removal efficiency remained constant at about 92.4 %. However, TP removal efficiency decreased from 90 % to approximately 80 % at COD concentrations of 1500–500 mg/l and to approximately 72 % at COD concentration of 250 mg/l. More than 70 % of TP was removed at all COD/P ratios, though the best COD/P ratios were 25, 16.6, and 11.8 with corresponding removal efficiencies of 87.3, 87.2, and 84.5 %, respectively. Previous studies on nutrient removal systems have determined the ratios at which high nutrient removal efficiencies can be achieved. One study of an SBR system with an input COD of 800 mg/l and TP of 68 mg/l demonstrated that at a COD/P ratio of 10:1, TP removal efficiency was 82 %

(Broughton et al. 2008). In our reactor, at a COD/P ratio of 11.8, an input COD of 750 mg/l, and a TP concentration of 60 mg/l, the removal efficiency was 84.5 %.

Conclusion

The novel anaerobic–anoxic/aerobic SBR described in this study had a good COD and nutrient removal efficiency and could successfully remove nitrogen and phosphorus compounds simultaneously. Given the improved nutrient removal when alternating anaerobic–anoxic/aerobic cycles, Phase 4 was identified as the most efficient phase. The key advantages of this new reactor include continuous influent, obviation of the need for sludge or wastewater reuse, lower space requirements through the use of compressed tanks, and the efficient simultaneous removal of phosphate and nitrogen.

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